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Chromatin Immunoprecipitation with Chelex

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Procedure

1. After counting cells (at least 5 X 10^7 or you will not have enough to sonicate), resuspend them in media containing 1% formaldehyde.
2. Rotate at room temp for 10 min (parafilm tubes or they will leak).
3. Quench by addition of 1 M glycine to final concentration of 0.125 M. Rotate at room temp for 5 additional minutes.
4. Pellet cells.
5. Rinse 2 times with PBS.
6. Resuspend cells in Nuclear Isolation Buffer. Leave on ice for 10 minutes with occasional inversion.
7. Pellet nuclei at 600 g for 7 min.
8. Resuspend nuclei in Nuclei Lysis Buffer to 10^8 nuclei per mL. Crosslinked nuclei can be kept at –80 degrees at this point, before sonication, but not after sonication. Determine sonication conditions for your cell type, and for your desired fragment sizes. (I used three 20 second pulses at power level 4 with 10 second pauses in between for HeLa and 293T cells) You must have at least 500 ul of lysed nuclei to be able to sonicate in an eppendorf tube, although I have sonicated up to 1200 ul in one tube. Make sure the sample gets equal sonication by ensuring that the sonicator tip is close to the bottom of the tube, and not touching sides. After sonication, immediately centrifuge sample for 15 min at 4 degrees to pellet nuclear crap. Take supernatant. For an individual IP reaction, 50 ul (or sonicated chromatin from 5 X 10^7 cells) was added to 1 mL IP dilution buffer (see below). Diluted chromatin was pre-cleared by addition of 25 ul of ProA beads overnight, rotating at 4 degrees. Pre-cleared diluted chromatin was separated from ProA beads and anti-serum was added (8 ul of mo20bR or 13 ul Tri20Br, 6 ul of H3 general) and immune complexes were allowed to form, rotating at 4 degrees for 4 hours. 20 ul of washed ProA beads were then added and incubation occurred for 1 hour, rotating at 4 degrees. Beads were then washed 8 times with 1 mL of RIPA buffer (Make RIPA buffer fresh with fresh sodium deoxycholate solution) and 1 time with 1 mL TE buffer. 100 ul of 10% Chelex solution (Bio-Rad) was then added to washed beads. Beads and Chelex solution were then incubated on 100 degree heat block for 10 min and allowed to cool to room temp. 1 ul of ProK was then added and bead and Chelex solution was incubated on 55 degree heat block for 1 hour. Eluate fraction was then separated. Beads and Chelex were washed with 100 ul of TE, which was added to previous eluate.

9. 1 % of this eluted fraction was used as for PCR amplifications
    9.1. To obtain input DNA, 5 ul of cleared sonicated chromatin was added to 300 ul of TE buffer. 15 ul of 5M NaCL was added, along with 1 ul of RNase A. This reaction was kept at 65 degrees overnight to reverse crosslinks. Then add 2 ul of ProK and digest for 4 hours at 55 degrees. Phenol:chloroform extract and resuspend DNA to 1 mL with TE. . Use 1 ul for PCR amplifications, which is 0.01%. 30 cycles were used for PCR amplifications.

Solutions

Nuclei Lysis Buffer

50 mM Tris-Cl pH 8.1
10 mM EDTA
1% SDS

RIPA Buffer

50 mM HEPES, pH 7.4
1 mM EDTA
1% NP-40
0.7% sodium deoxycholate
500 mM LiCl

Nuclear Isolation Buffer

150 mM NaCl
10 mM Hepes pH7.4
1.5 mM MgCl2
10 mM KCl
0.5% NP-40 (This may need to be decreased, depending on cell type)
0.5 mM DTT
Protease inhibitors